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# Spatial variability in mycorrhizal hyphae and nutrient and water availability in a soil-weathered bedrock profile

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**Abstract**

We documented the spatial distribution, abundance and molecular diversity of mycorrhizal hyphae and physical and chemical properties of soil-weathered bedrock in a chaparral community that experiences seasonal drought. Because plants in this community were known to rely on bedrock-stored water during the summer, the data were used to evaluate the potential role of mycorrhizal hyphae in accessing bedrock-stored water during summer drought. The granitic bedrock was characterized by factures filled with a disaggregated, sandy loam that acted as conduits for water, and matrices composed of soil-weathered granite that retained the fabric and structure of rock. Mycorrhizal hyphae of six ectomycorrhizal taxa (from the Basidiomycota and Ascomycota), and arbuscular mycorrhizal hyphae (Zygomycota) were recovered from both fracture and matrix compartments to depths greater than 200 cm. Our findings also indicated a potential linkage between the abundance of Ascomycete hyphae, substrate physical (bulk density) and chemical properties (total N, N:P, Ca:Mg), and bedrock moisture content, as well as spatial patterning between hyphae and resources at a scale of 25–45 cm. Such linkages suggest that mycorrhizal fungal hyphae may be part of an adaptive mechanism that enables chaparral plants to survive seasonal drought.

*Abbreviations:* AM – arbuscular mycorrhizae; EM – ectomycorrhizae; ITS – internal transcribed spacer

## Introduction

Plants rely on water stored within the substrate to meet transpirational demands during seasonal drought (Canadell and Zedler, 1995). Such is the case in Mediterranean-type climates, where wet winters recharge substrates but virtually no precipitation falls during the summer. Under these conditions, the survival and growth of many plants is reliant on morphological and physiological traits that either control water loss or increase water uptake (Parsons et al., 1981). Most notably, many trees and shrubs in Mediterranean climates have deep and extensive root systems that exploit soil-stored water during summer drought (Hellmers et al., 1955; Schenk and Jackson, in press).

In foothills where the soils are relatively thin, the roots of these woody plants commonly penetrate deeply into the weathered bedrock zone by following fractures (Hellmers et al., 1955; Stone and Kalisz, 1991). In these areas, the store of plant-available water within weathered bedrock often exceeds that of the soil on a whole volume basis (Flint and Childs, 1984; Jones and Graham, 1993) because these strata are considerably thicker than the overlying soil (Hubbert et al., 2001b). For that reason, weathered bedrock constitutes a key water-holding substrate in chaparral

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and forest ecosystems (Anderson et al., 1995; Arkley, 1981; Sternberg et al., 1996; Zwieniecki and Newton, 1996). Within the bedrock, roots are confined to fractures. However, water is extracted from the weathered rock matrix as well as adjacent to the fractures (Hubbert et al., 2001a). Since fractures can be spaced up to 50 cm apart, and unsaturated flows in coarse materials are exceptionally slow ($<10^{-3}$ cm $h^{-1}$; Jury et al., 1991), the question arises as to how the water held in the rock matrix might be obtained by plants.

A potential, but thus far unexplored, mechanism of adaptation to water stress in weathered bedrock may include mycorrhizal fungi. Mycorrhizal fungi constitute one of the water (and nutrient) acquisition strategies available to the vast majority of terrestrial plants (Allen, 1991). These fungi serve as conduits from the soil to plant roots, and transfer water (Augé, 2001; Duddridge et al., 1980) and inorganic and organic nutrients to the host plant using a network of extraradical (external) hyphae (Allen, 1991). These hyphae may extend for more than 2 m from the root surface (Fogel, 1983), and are sufficiently small in diameter as to penetrate microfractures (Duddridge et al., 1980). However, few studies have considered the role of mycorrhizae in acquiring and transferring water from weathered bedrock to the host plant (Hubbert et al., 2001a,b).

In this study, we documented the spatial distribution, abundance and diversity of mycorrhizal and non-mycorrhizal extraradical hyphae, and the scale of patterning between bedrock properties and the fungal community using spatial autocorrelation. We hypothesized that if mycorrhizal hyphae were common in bedrock matrices and there was a non-random spatial pattern of hyphal abundance and diversity with the distribution of resources, then plants associated with these mycorrhizal taxa may be buffered from edaphic stresses. Alternatively, if hyphae were absent from matrices, limited to point sources such as fractures, or randomly associated with resources, then mycorrhizal benefits to the host plants may be limited. We tested this hypothesis in a chaparral community in southern California, where plants are known to rely on bedrock-stored water during summer drought (Hubbert et al., 2001a; Sternberg et al., 1996).

## Materials and methods

### *Environmental setting*

The study site is on a nearly level hill summit (1150 m a.s.l.) in the foothills of the San Jacinto Mountains in southern California, about 130 km east of Los Angeles (33°43′N, 117°10′E). The climate is typically Mediterranean (35 °C/14 °C summer; 18 °C/3 °C winter), where rainfall occurs from November to March and little or no rainfall is typically recorded from April to October. Measured precipitation averages 550 mm per annum (National Oceanographic Atmospheric Administration; http://lwf.ncdc.noaa.gov/oa/ncdc.html). Correspondingly, maximum water stress occurs during late summer or early fall when ambient temperatures are high and little water is available for plant uptake.

The vegetation is characterized by a mosaic of patches dominated either by *Adenostoma fasciculatum* Hook and Arn. (chamise; Rosaceae) or *Arctostaphylos glandulosa* Eastw. (Eastwood manzanita; Ericaceae), with a mix of other woody species including *Ceanothus greggi* A. Gray (cupleaf ceanothus; Rhamnaceae), species of *Salvia* (sage; Lamiaceae) and *Eriogonum* (buckwheat; Polygonaceae), in the openings. No fires have burned in the area for at least 20 years. Roots of *Adenostoma* may be colonized by ecto- (EM) and arbuscular mycorrhizae (AM) and *Ceanothus* by AM, whereas *Arctostaphylos* forms arbutoid mycorrhizae (Allen et al., 1999; Zak, 1976). The fungi of arbutoid mycorrhizae are often the same fungal species that form EM associations. EM fungi include representatives from the Basidiomycota and Ascomycota, whereas the AM fungal associates are solely from the Zygomycota (Allen, 1991).

The pedology of the site, summarized here, is detailed in Sternberg et al. (1996) and Graham et al. (1997). The bedrock is tonalite of the late Mesozoic Peninsular Ranges batholith, one of the largest granitic bodies in the United States (Hill, 1988). It is weathered to several meters in depth, yielding a regolith that retains the fabric and structure of rock, but is sufficiently friable so it can be excavated with a pick and shovel and individual chunks can be crushed by hand. We do not consider it to be saprolite because the feldspars and micas are not thoroughly weathered to clay minerals, and it does not meet the definition of Bates and Jackson (1987) which specifies ‘a soft, earthy, typically clay-rich, thoroughly decomposed rock. . . ’ Nevertheless, this weathered bedrock has many properties in common with soils, including appreciable plant avail-

able water capacity (0.14 $m^3$ $m^{-3}$; Sternberg et al., 1996) and saturated hydraulic conductivity (3–4 cm $h^{-1}$; Graham et al., 1997). The soils are coarse-loamy, mixed, superactive, mesic Typic Xerorthents and are about 35 cm thick over the weathered bedrock, which is generally 4 m thick to hard bedrock.

*Soil sampling*

A trench was excavated in April 1993 to expose a regolith (soil + weathered bedrock) cross-section 2.4 m long and 2.2 m deep; depth was restricted by the underlying hard bedrock. A morphological description was made on one end of the trench face (Soil Survey Division Staff, 1993) and samples were taken from each of the identified morphologic horizons at 25-cm horizontal intervals. Within each morphologic horizon, stratified sampling was employed to take into account the heterogeneity created by the juxtaposition of bedrock matrices and fractures (see Figure 1). In particular, samples were designated as originating from fracture fill material (hereafter 'fracture') or weathered rock matrix (hereafter 'matrix'), the material between the fractures. The profile was also sampled from the bottom upward to avoid contamination from the overlying horizons. Samples were transported to the laboratory in sealed plastic bags.

*Soil analyses*

Bulk samples were air dried, crushed to break down aggregates, and sieved to remove rock fragments >2 mm in diameter. All laboratory analyses were made on the <2 mm fraction unless otherwise noted. Particle size distribution was determined by the pipette and sieving method (Gee and Bauder, 1986). Bulk density was measured using paraffin-coated clods (Blake and Hartge, 1986). Total carbon (C) and nitrogen (N) were measured by dry combustion (Nelson and Summers, 1982), and the C was assumed equal to organic C since no carbonates are present in the soil and rock materials. Plant-available phosphorus (P) was determined using a dilute acid–fluoride extraction followed by spectrophotometric determination (Olson and Sommers, 1982). The pH of soil and weathered bedrock was measured using a 1:1 soil:water ratio. Exchangeable cations were extracted with 1 M ammonium acetate solution and measured by atomic absorption spectrometry (Thomas, 1986).

*Extraction of hyphae*

Extraradical hyphae were extracted from duplicate aliquots of soil from each sample point in the soil profile. For an individual sample, 5 g of soil were extracted in 200 ml of sodium hexametaphosphate (39.5 g $L^{-1}$) for 1 h, washed through a 250 $\mu$m mesh, then resuspended in 300 ml of distilled water, left to settle for 15 s, and decanted through a 28-$\mu$m sieve (after Frey and Ellis, 1997). Hyphae were then rinsed out of the sieve into 50 mL of distilled water and allowed to stand for 30 s. Two sub-samples (each 3 mL) were taken from the sample and filtered through individual 1.2-$\mu$m membranes. The membranes were mounted on slides with Permount® and viewed using a compound microscope (×200–400 magnification). The remaining hyphae were collected over a 28-$\mu$m sieve, rinsed with double-distilled water, concentrated into 1 mL of double-distilled water, and freeze-dried prior to DNA extraction.

*Identification of fungi*

DNA was extracted from approximately 25 mg of hyphae per sample using the Qiagen DNeasy kit. For PCR amplification, equal amounts of DNA (20–25 $\mu$L) and a PCR mixture of 20 nmol dNTP, 20 pmol of primer (synthesized by Sigma Genosys), 1 × PCR buffer, and 2.5 units *Taq*-polymerase were mixed. Primer combinations were: ITS1-F/ITS4-B (Basidiomycota-specific), or ITS1-F/ITS4 (fungal specific). Thermocycling conditions consisted on an initial hold at 93 °C for 3 min followed by 30 cycles of 95 °C (30 s), 55 °C (2 min), 72 °C (2 min), and a final hold of 72 °C for 10 min. Controls using double-distilled water were used to check for contamination. The amplification reactions were separated on 3% agarose gels to check for PCR products.

Samples with single amplification products, i.e., all DNA from one source, were analyzed by restriction fragment length polymorphisms (RFLP). The amplified PCR product was digested with two endonucleases, *Hinf*1 and *Taq*1, following manufacturer's directions. The resultant restriction fragments were size fractionated on 3% agarose gels and stained, and the patterns imaged and analyzed using Kodak DC290 image capture and '1-D' analysis software; these patterns were compared with reference material. The majority of samples produced only one PCR product. However, for samples with dual amplification, i.e., two or more PCR products that potentially came from two

or more fungal species within the sample, individual bands were excised from the gel, and DNA was eluted from each band using a QIAEx gel extraction kit. The extracted DNA was amplified by PCR and subjected to RFLP as described previously.

Any samples with fragment patterns that did not correspond with published data were sequenced. PCR products were cleaned using a QIAquick PCR clean up kit (Qiagen). A QuickStart dye terminator cycle sequencing kit (ResGen/ Beckman) was used to prepare samples, and electrophoresis and data collection were undertaken in a CEQ8000 sequencer (Beckman Coulter). The identity of each band was determined by comparisons with sequence alignment files in Bruns et al. (1998).

*Enumeration of fungal hyphae and diversity*

Fifty randomly located fields of view on each membrane were scored using the gridline intercept method for total hyphal abundance (mycorrhizal plus non-mycorrhizal) using a compound microscope (×400 magnification). Scores were converted to meters of hyphal length per gram dry weight of soil (Tennant, 1975). Scores were then subdivided into AM hyphae and 'other fungi', which included mycorrhizal and non-mycorrhizal taxa. We differentiated AM hyphae in this manner since they possess morphological features that are distinct from those of other mycorrhizal types (Allen, 1991), and because AM fungi are less amenable to molecular analyses than members of either the Basidiomycota or Ascomycota.

To calculate the hyphal length of Basidiomycota, Ascomycota and non-mycorrhizal taxa within the sample, we used a proxy of abundance based on both microscope counts and RFLP data. For each sample, the length of 'other' hyphae (= total length – AM length) was allocated as Basidiomycota, Ascomycota or non-mycorrhizal based on the molecular identification of the sample. Because most samples contained only one PCR product, the molecular identity of the hyphae was considered to represent the identity of the hyphae within the sample. These data were then sorted and pooled as Basidiomycota and Ascomycota (mycorrhizal fungi), or non-mycorrhizal.

*Statistical analyses of hyphal abundance and soil properties*

In each of the fracture and matrix, the abundance of each mycorrhiza type and non-mycorrhizal hyphae were analyzed between strata using one-way analysis of variance (ANOVA). Data were not transformed prior to ANOVA since all data sets were homoscedastic (by Kolmogorov–Smirnov test, $P > 0.05$). Significant differences among mean values were further analyzed by Fisher's least significant difference (LSD) test. These analyses were undertaken using SYSTAT (v.5).

*Spatial analyses of biotic and pedologic traits*

We used autocorrelation to explain the variation in biotic traits (fungal diversity, abundance) across pedological space (trench depth, fracture versus matrix). This approach assumes that the autocorrelation calculations describe the similarity of objects over the whole surface (trench), and variables are stochastically independent from one another (Isaaks and Srivastava, 1989; Legendre, 1993). Such patterns of spatial dependency and partitioning among distance classes (40-cm intervals in this study) were calculated using Moran's **I**, which is structured to be similar to the conventional statistical idea of positive ($\mathbf{I} > 0$, localized patterning), negative ($\mathbf{I} < 0$, random distribution) or no correlation among variables ($\mathbf{I} = 0$, patchwork pattern). Correlograms for each trait were plotted on the ordinate and against depth classes, and calculated **I** values were tested for significance using the null hypothesis that variables were independent but each of them was autocorrelated ($H_o$: $\mathbf{I} = 0$, where '0'= $[-1 / (n - 1)]$, and $n$ is the number of variables).

A 'partial regression approach' was used to divide the variance between the spatial and biotic components within the trench into four identifiable fractions using the methodology described in Legendre (1993). These four fractions were as follows: (i) non-spatial biotic variation ($r^2{}_1$,); (ii), spatially structured biotic variation (soil × fungal interactions; $r^2{}_2$); (iii) non-biotic spatial variation in soil properties (= $r^2{}_3 - (r^2{}_1 + r^2{}_2$,), and (iv), variation that cannot be explained be either spatial or biotic components ($1 - r^2{}_3$). Partial regression of single variables or constrained ordination (partial redundancy analysis) of multivariate data sets was used to calculate $r^2$ values. Multiple regression (backwards elimination, 999 permutations) and Spearman Rank correlation coefficients were used to confirm the contributions of significant variables to the observed spatial variation. Statistical analyses were undertaken using the 'R' package (v.4) for spatial analyses, and 'Permute!' (v3.4) for multiple regressions

*Table 1.* Chemical and physical properties of the soil-weathered granitic bedrock profile in the San Jacinto Mountains in southern California. Standard errors are given in parentheses below each mean

| Horizon | Depth (cm) | Gravel (%)[†] | Sand (%) | Silt (%) | Clay (%) | Bulk Density (g cm$^{-3}$) | Organic C (%) | Total N (%) | P (ppm) | Exchangeable cations ($\mu$mol kg$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Ca | Mg | K | Na |
| A | 0–5 | 18 | 75 | 17 | 8 | nd[‡] | 5.01 | 0.257 | 12.64 | 17.0 | 1.9 | 0.7 | <0.1 |
| | | (0.6) | (1.18) | (0.6) | (0.4) | | (0.31) | (0.072) | (0.51) | (0.58) | (0.19) | (0.11) | |
| AC | 5–20 | 20 | 75 | 19 | 6 | 1.35 | 1.05 | 0.063 | 7.54 | 6.3 | 1.1 | 0.8 | <0.1 |
| | | (0.6) | (0.12) | (0.6) | (0.3) | (0.16) | (0.14) | (0.035) | (0.39) | (0.36) | (0.14) | (0.13) | |
| C | 20–35 | 38 | 72 | 20 | 8 | 1.48 | 0.56 | 0.036 | 4.08 | 5.2 | 1.2 | 0.6 | <0.1 |
| | | (0.9) | (1.21) | (0.5) | (0.4) | (0.17) | (0.11) | (0.002) | (0.29) | (0.32) | (0.15) | (0.12) | |
| Cr/ C3[¶] | 35–85 | Cr 51 | 76 | 18 | 6 | 1.72 | 0.27 | 0.016 | 7.31 | 4.3 | 1.3 | 0.5 | <0.1 |
| | | (1.0) | (1.24) | (0.5) | (0.3) | (0.19) | (0.07) | (0.008) | (0.38) | (0.29) | (0.16) | (0.10) | |
| | | C 32 | 73 | 20 | 7 | | 0.27 | 0.016 | 9.71 | 5.0 | 1.7 | 0.4 | <0.1 |
| | | (0.8) | (1.22) | (0.6) | (0.3) | | (0.06) | (0.010) | (0.44) | (0.31) | (0.18) | (0.09) | |
| Cr | 85–220 | F[§] 50 | 84 | 14 | 2 | 2.34 | 0.08 | 0.004 | 19.29 | 2.9 | 1.0 | 0.1 | 0.2 |
| | | (1.0) | (1.33) | (0.5) | (0.2) | (0.22) | (0.04) | (0.001) | (0.62) | (0.24) | (0.13) | (0.04) | |
| | | M 46 | 77 | 18 | 5 | 2.08 | 0.40 | 0.013 | 5.90 | 6.9 | 3.8 | 0.2 | 0.3 |
| | | (0.9) | (1.25) | (0.6) | (0.3) | (0.21) | (0.09) | (0.002) | (0.34) | (0.37) | (0.27) | (0.06) | |

[†]Gravel content calculated on whole soil basis; sand, silt and clay as percentage composition in <-2 mm fraction.
[‡]nd – not determined.
[¶]Horizon consisted of an intricate intermingling of Cr- and C-type materials. Bulk density is for these materials combined. All other data are reported individually for the two material types, which were analyzed separately.
[§]Weathered bedrock matrix blocks (M) and the material filling the joint fractures (F) were sampled and analyzed separately.

(http://www.fas.umontreal.ca/BIOL/Casgrain/en/siteOutline.html).

## Results

### *Soil and bedrock characteristics*

The soil at the site, an Entisol with sandy loam texture, was underlain at a shallow depth by granitic bedrock (Table 1). The bedrock was weathered sufficiently soft that it could be excavated with a pick and shovel and chunks of it were easily crumbled to loose grains with bare hands. Within the bedrock, joint fractures separated intact matrix material into blocks 40–80 cm across (Figure 1a). The fractures were not empty voids but were filled with a disaggregated, sandy loam material. Roots were concentrated in these fractures, while the matrix was devoid of field-observable roots (Figure 1b). In the weathered bedrock matrix, mineral grains retained their original orientations relative to each other, but microfractures 100–500 $\mu$m in width permeated the fabric (Figure 1c). Chemical weathering produced dissolution pits of capillary size ($\leq$10 $\mu$m) in feldspar grains (Figure 1d).

The soil-weathered bedrock profile was coarse textured, with sand contents >70% and clay contents <10% throughout (Table 1). The coarsest textures were in the Cr material (84% sand, 2% clay). Bulk density increased with depth and was much higher in the weathered bedrock than in the soil (Table 1). Fracture materials also had a substantially lower bulk density than the weathered rock matrix.

Carbon and total N were highest within the soil A horizon (5 and 0.25%, respectively) and decreased with increasing depth; these resources were also present in much higher concentrations within the fracture material than the matrix (Table 1). Substrate pH values ranged from 5.5 to 6.5 and overall, levels within the weathered matrix were lower than those in the soil. Extractable P was highest in the A horizon (13 ppm) and in the Cr matrix material (19 ppm). Exchangeable Ca was highest in the A horizon (17 $\mu$mol kg$^{-1}$) and < 7 $\mu$mol kg$^{-1}$ in the remainder of the substrate. Exchangeable Mg was < 2 $\mu$mol kg$^{-1}$, except in the Cr fracture material, where it was 3.8 $\mu$mol kg$^{-1}$. Exchangeable K ranged from 0.8 to 0.1 cmol$^{+}$ kg$^{-1}$, and generally decreased with increasing depth. Exchangeable Na was barely detectable throughout the profile.

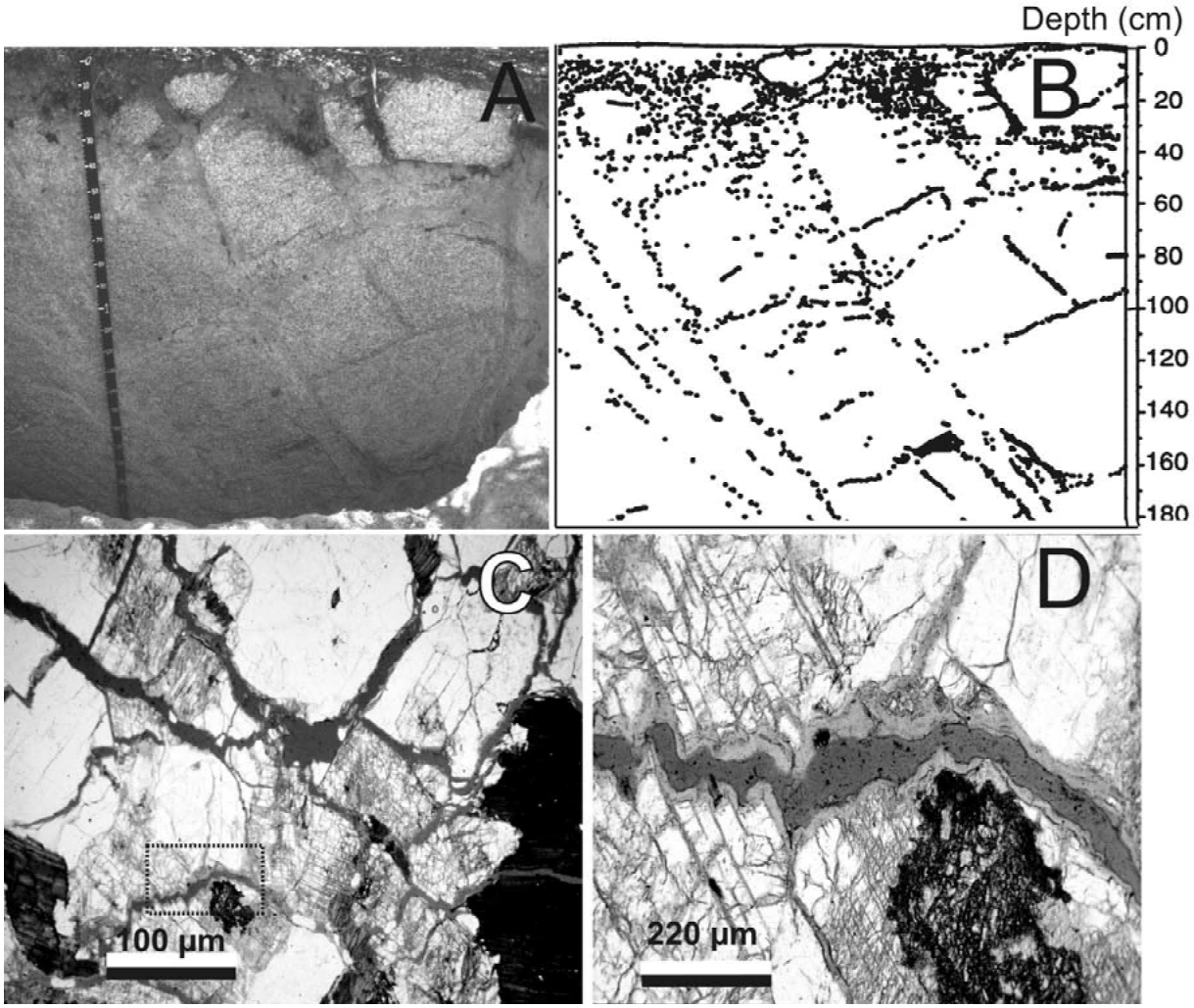


*Figure 1.* (a) Photograph showing cross-section through soil and weathered bedrock in the San Jacinto Mountains, California, and (b) Diagrammatic representation of the distribution of fine roots within the same vertical profile. (c,d) Photomicrographs at low and high magnification respectively of thin sections of weathered bedrock illustrating porosity and microfractures. Square area outlined by dashed lines on (c) is shown in detail in (d).

The chaparral vegetation depleted soil and bedrock water throughout the spring and summer, with water depletion clearly extending to depths of 377 cm (Figure 2). Gravimetric water loss at the 25 cm soil depth was less than 2% from April to September because most of the soil moisture was lost before the April measurement as evapotranspiration losses increased with warming March temperatures. For both the Cr/C horizon (35–85 cm) and the Cr horizon (85–220 cm), seasonal water depletion from April to September averaged 5.5%. From 220 cm to a depth of 377 cm, the decrease in bedrock (Cr) water content was less, averaging only 2.2% from April to September (Figure 2).

### *Distribution, abundance and diversity of fungal hyphae*

Fungal hyphae were distributed throughout the bedrock profile (Figure 3). Within both the fractures and the matrices, hyphal abundance was significantly higher within the upper soil and bedrock layers (0–16 cm depth, A and AC horizons), and declined with depth ($P < 0.05$). Hyphal abundance declined markedly at depths below 73 cm (Figure 3). In addition, the overall abundance of hyphae did not differ

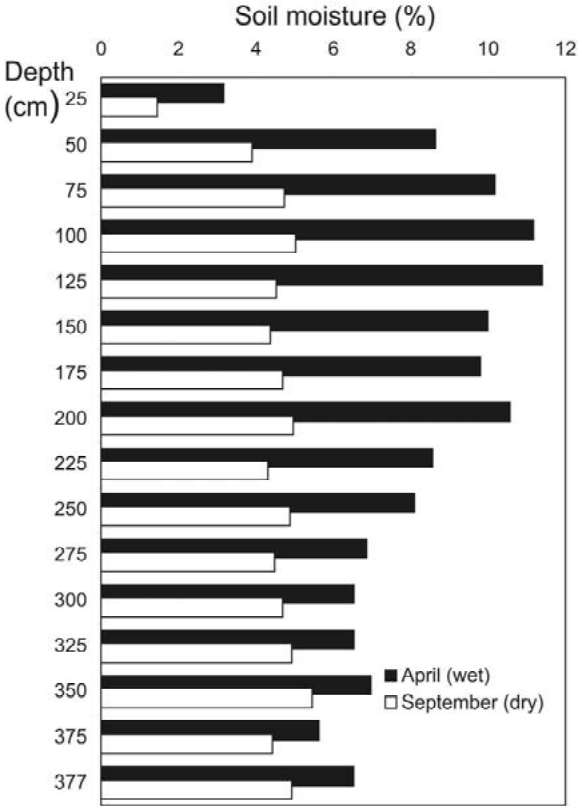


*Figure 2.* Soil and bedrock water content during the wet season (April) and after a period of summer drought (September) with vertical depth in the San Jacinto Mountains.

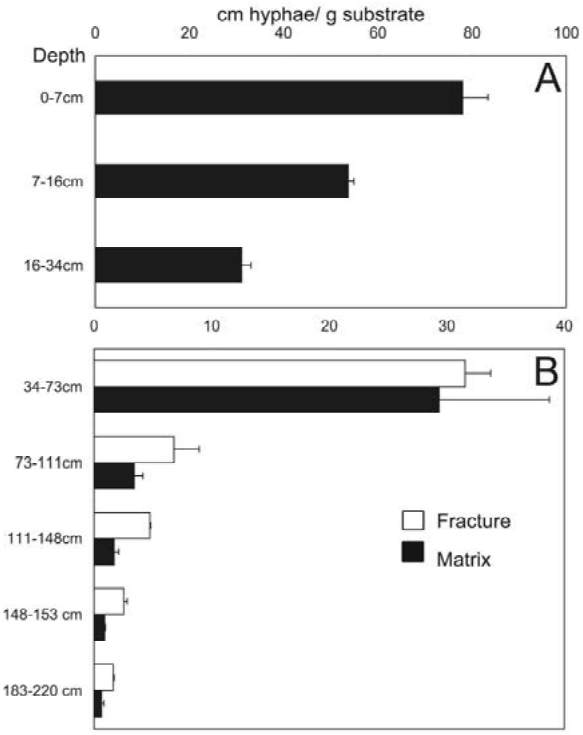


*Figure 3.* Distribution and abundance of mycorrhizal fungal hyphae and fine roots with vertical depth in the weathered bedrock profile. (a) Abundance of fungal hyphae (all mycorrhizal and non-mycorrhizal) between 0 and 34 cm depth, and (b) abundance of fungal hyphae within the matrix and fractures of weathered bedrock between 34 and 220 cm depth. Horizontal bars indicate the standard error of the mean.

significantly within the matrix and fractures between 34 and 73 cm ($P > 0.05$). At greater depths, however, hyphae were more abundant within the fractures than within the bedrock matrix.

Nine distinct RFLP types were present within the profile (Figure 4); most of these were identified to some level of taxonomic resolution. Four taxa accounted for 76% of the hyphal community within the bedrock: *Rhizopogon* and *Pisolithus* were the most abundant Basidiomycota, whereas *Wilcoxina* and *Cenococcum* represented the most abundant Ascomycota (Figure 4a). Ascomycota, including *Wilcoxina*, are common in fire-prone nature environments, such as the chaparral (Baar et al., 1999). The depth distribution of RFLP-taxa illustrates maximum species richness ($n = 6$ taxa) within the upper soil profile (0–5 cm) as well as mycorrhizal taxa that were restricted to this layer (*Laccaria*, *Hebeloma*) (Figure 4b). The depth distribution of mycorrhizal taxa within the bedrock also indicated the presence of recurring ectomycorrhizal taxa (*Rhizopogon*, *Cenococcum*, *Wilcoxina*) and the Zygomycota. In particular, *Rhizopogon* and *Cenococcum* were recovered from within the matrix materials. In addition, three unknown fungal species (or 13% of samples) were detected. Because these samples did not conform to known mycorrhizal taxa, they were classified as non-mycorrhizal.

### *Spatial patterning of soil and fungal variables*

Spatial (soil) attributes alone accounted for 31% of the observed variation within the bedrock profile (Table 2). Most of this was associated with the percentage of sand, gravel, silt and clay, bulk density, and concentrations of Na, K and N within the substrate (Table 3). In addition, the contribution from the biotic (fungal) and interaction between soil and fungal traits accounted for ∼42% of the total variance (Table 2). However, 20% of the variance within the profile could not be explained by either fungal or soil variables assessed in this study. It is possible that other (untested)

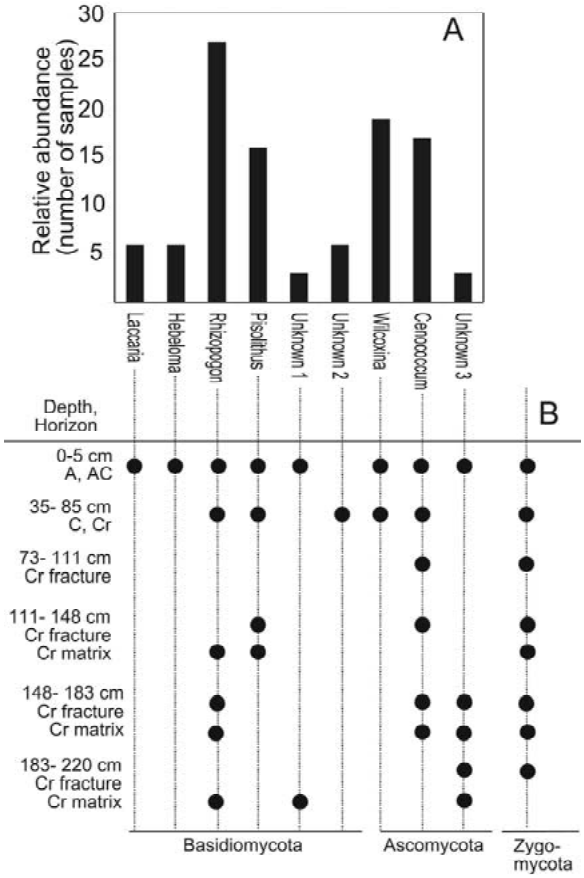


*Figure 4.* (a) The relative abundance of each taxa of mycorrhizal fungi (Basidiomycota, Ascomycota) identified by molecular analyses, as well as three unknown species that represent non-mycorrhizal taxa. (b) Distribution of hyphae by taxa for arbutoid and ectomycorrhizae Basidiomycota and Ascomycota, and for arbuscular mycorrhizae (Zygomycota) as a group, with vertical depth.

*Table 2.* Summary of significant Moran's **I** for soil variables and range of autocorrelation with fungal hyphal abundance. *P* values derived from analyses are given in parentheses after each variable

| | **I** | Range (cm) |
|---|---|---|
| First-order variables | | |
| % Sand | −1.36 (0.009) | 148–197 |
| % Silt | −1.08 (0.045) | 197–248 |
| % Clay | −1.34 (0.014) | 197–248 |
| % Gravel | 0.20 (0.030) | 0–15 |
| | −1.26 (0.003) | 45–75 |
| Bulk Density | 0.88 (0.035) | 25–45 |
| | −1.43 (0.001) | 197–248 |
| Na | 0.79 (0.014) | 25–45 |
| | −0.86 (0.023) | 197–248 |
| K | 1.03 (0.017) | 25–45 |
| | −1.59 (0.001) | 197–248 |
| Total N | 0.24 (0.042) | 25–45 |
| | −0.72 (0.003) | 197–248 |
| Second-order variables | | |
| N:P | 0.56 (0.046) | 0–15 |
| | −0.95 (0.013) | 197–248 |
| C: N | −0.98 (0.042) | 144–197 |
| C: P | −0.78 (0.032) | 197–248 |
| Ca:Mg | 0.77 (0.002) | 0–15 |
| | −1.18 (0.019) | 197–248 |
| Third-order variables | | |
| Water × N:P | 0.16 (0.007) | 0–15 |
| (summer) | −0.98 (0.007) | 197–248 |
| Water × Ca:Mg | 0.00 (0.001) | 45–145 |
| (summer) | | |

variables are important determinants of spatial patterning within this system, such a host phenology, and mycorrhizal longevity and turnover.

Autocorrelation analyses and correlograms of **I** demonstrated significant departures from randomness and two scales of patterning in soil variables at the first, second and third order. These data are summarized in Table 3. Percentage gravel, bulk density, Na, K, and total N, all showed two ranges of significant autocorrelation. These data demonstrated localized patterning near the surface ($\mathbf{I} > 0$), with a scale of 24.5 cm. Similarly, the second-order relationships revealed localized patterning ($\mathbf{I} > 0$) for N:P, Ca:Mg and Na: K, and third-order relationships between soil moisture (summer) with N:P and Ca:Mg. We also observed an overall trend towards random distributions at depth ($\mathbf{I} < 0$), particularly between 197 and 248 cm. Backwards elimination regression retained total N, N:P, Ca:Mg, and the interaction between these variables and soil moisture content during summer as determinants of mycorrhizal hyphal distribution. In all correlograms, $\mathbf{I} = 0$, or patchy distribution of soil factors, occurred at ∼80 cm depth. This corresponds, in part, with the region of intermingled Cr and C type materials (Table 1).

Spearman rank correlation coefficients demonstrated the relationships between the spatially significant variables from autocorrelation (Table 3) with the abundances of each type of mycorrhizal hyphae within the depth profile (Table 4). Apart from bulk density, K, and N, the remaining soil physical and chemical

*Table 3.* Partitioning of the variation between biotic and spatial (environmental) components properties of the soil-weathered granitic bedrock profile in the San Jacinto Mountains in southern California

| Component | Variance explained (%) |
|---|---|
| (1) Non-spatial biotic variance ($r^2{}_1$) | 20.77 |
| (2) Spatially-structured biotic variation ($r^2{}_2$) | 27.01 |
| (3) Non-biotic (soil) spatial variation ($r^2{}_3 - (r^2{}_1 + r^2{}_2)$ | 31.63 |
| (4) Unexplained variation ($1 - r^2{}_3$) | 20.59 |

parameters were not significantly correlated with the abundance of mycorrhizal hyphae. Thus, these factors were autocorrelated with soil depth but were not significantly associated with the abundance of hyphae. Conversely, the abundance of mycorrhizal hyphae was negatively correlated with bulk density. For this reason, hyphae likely traversed the path of least resistance through microfractures within the granitic bedrock (Graham et al., 1997; Sternberg et al., 1996) that were created by chemical weathering (Figure 1). These analyses also strongly indicated that second- (N:P, Ca:Mg, Na:K) and third-order variables (moisture $\times$ N:P or Ca:Mg) were highly correlated with the abundance and diversity of Ascomycota within the profile ($P < 0.001$). Conversely, we did not detect any significant relationship between the abundance of hyphae of Basidiomycota, Zygomycota, or non-mycorrhizal fungi and these soil variables.

## Discussion

The Mt San Jacinto profile indicates a potential causal linkage between mycorrhizal hyphal abundance, substrate physical (bulk density) and chemical properties (N:P, Ca:Mg), and bedrock moisture content. Because hyphae tend to localize in resource- rich patches (Bending and Read, 1995), the most parsimonious explanation is that mycorrhizae may be part of an adaptive mechanism that facilitates water uptake from bedrock sources to the host plant. Field studies have examined at length the adaptive importance of the patterns of rooting depth, lateral distribution, branching and extent of root penetration into fractures as contributors to water acquisition from bedrock (e.g., Canadell and Zedler, 1995; Hellmers et al., 1955; Sternberg et al., 1996; Parson et al., 1981). However, few studies have considered the potential role of mycorrhizae in this process (Hubbert et al., 2001a,b). In our study, the Mt San Jacinto profile was sufficiently structurally diverse, i.e., with a variety of individual structures, to demonstrate spatial patterning and complex interactions between fungal and edaphic components.

Spatial patterning or structural patches across the profile (horizontal heterogeneity) were largely the result of spatial aggregation of hyphae and nutrients. The difference in hyphal abundance (and fine roots; Sternberg et al., 1996) between fracture and matrix is an obvious demonstration of this patterning. Nevertheless, the presence of hyphae within the bedrock matrix substantiates their potential for water acquisition, even at depth ($>$ 111cm). On the vertical scale, spatial patterning incorporated the recovery of hyphae from the upper (A) horizon to the weathered bedrock, as well as the small-scale patterning of fungi and resources within the A or AC horizons (patch size 24–40 cm), and the trend towards large-scale patterning and/or heterogeneity with depth (Cr horizons). In both instances, however, spatial patterning was coincident with resource availability, including substrate moisture over summer.

One possible explanation for this trend may be the root distribution patterns of the dominant chaparral shrubs. Sub-shrubs, forbs and grasses with fibrous root systems (*Salvia*, *Eriogonum*), and woody shrubs with predominantly lateral root growth (*Ceanothus*) typically colonize the upper soil horizons (to 1 m; Hellmers et al., 1955). The fungi of these plants might utilize localized substrates or those in patches or gaps at the fracture–matrix boundary (Fitter, 1994; Hodge et al., 1998). Small-scale heterogeneity may also influence seedling establishment (Stark 1994), and the distances over which grasses, herbs, shrubs and young trees are likely to interact (Tilman, 1989). Conversely, woody shrubs with coarse roots (*Adenostoma*, *Arctostaphylos*) have the greatest ability to extend their roots deep into fractures (3 m; Hellmers et

*Table 4.* Spearman Rank correlation coefficients between mycorrhizal fungal types and soil variables identified from the autocorrelation analyses as being significant contributors to spatial patterning within the weathered bedrock profile

| Variable | Mycorrhizal fungi | | | Non-mycorrhizal fungi |
|---|---|---|---|---|
| | Basidio† | Asco. | Zygo. | |
| % Silt | −0.24 | 0.36 | −0.41 | 0.58 |
| % Clay | −0.25 | 0.37 | −0.41 | −0.42 |
| % Gravel | 0.14 | −0.46 | −0.11 | 0.28 |
| Bulk Density | 0.64 | **−0.82*** | 0.32 | 0.42 |
| Na | 0.49 | −0.49 | 0.14 | 0.04 |
| K | −0.75 | **0.85*** | −0.11 | −0.46 |
| Total N | −0.57 | **0.78*** | −0.25 | −0.37 |
| N:P | −0.59 | **0.84*** | −0.29 | −0.47 |
| C:N | 0.68 | −0.48 | −0.14 | −0.09 |
| C:P | −0.55 | 0.76 | −0.39 | −0.38 |
| Ca:Mg | −0.67 | **0.87*** | −0.28 | −0.50 |
| Na:K | 0.75 | **−0.86*** | 0.11 | 0.44 |
| Water × N:P | 0.57 | **−0.78*** | 0.32 | 0.46 |
| Water × Ca:Mg | 0.67 | **−0.89*** | 0.21 | 0.68 |

†Basidio, Basidiomycota; Asco, Ascomycota; Zygo, Zygomycota.
*bold, denotes significant at $P < 0.01$.

al., 1955) and thus induce spatial variation on a larger scale. These species may also depend on hydraulic lift from deep reservoirs, as well as water from bedrock-derived sources, to satisfy the plant demands for water. In this manner, the two different patterns of rooting, mycorrhizal colonization and hyphal distribution may result in two different scales of resource utilization.

A second explanation may be that the individual fungal taxa in the profile differ in their ecophysiological capacities. For example, the fungal taxa identified within the hyphal community were capable of forming extensive hyphal networks and rhizomorphs for the transfer of resources, especially water (*Rhizopogon*, *Pisolithus*; Agerer, 1987–1997; Duddridge et al., 1980), tolerating desiccation (*Rhizopogon*, *Wilcoxina*; Taylor and Bruns, 1999), or acquiring organic N (*Hebeloma*, *Cenococcum*, *Rhizopogon*; Abuzinadah and Read, 1986, 1989). Because these fungi form both ecto- or arbutoid mycorrhizae, they were likely associated with the deep-rooted *Adenostoma* and *Arctostaphylos*. Such functional differentiation among taxa in concert with their vertical and horizontal distribution might contribute to the spatial patterning of resource acquisition, especially for those taxa within matrix materials (*Rhizopogon*, *Cenococcum*). In addition, such relationships may help to explain, at least in part, the differential survival of *Arctostaphylos* and *Ceanothus* during severe drought (Parsons et al., 1981; Schlesinger and Gill, 1980).

While spatial (dis)similarity evaluated the distinctiveness of hyphal communities within all vertical strata, correlation and autocorrelation analyses examined the factors contributing to this pattern. Our findings indicate that the hyphal distribution recorded in Mt San Jacinto was not simply related to the physical constraints of the bedrock, even though a causative negative relationship existed between bulk density and hyphal abundance (see Table 4). Moreover, such patterns of hyphal abundance could not be explained fully by the effects of an individual variable, including the moisture content of the weathered bedrock. On the contrary, much of the observed variance came from the interactions between certain combinations of edaphic factors and the fungal biota. For example, second- (N:P, Ca:Mg) and third-order interactions (water × N:P) provided the best predictors of hyphal abundance. It follows that bedrock systems are comprised of biotic and abiotic factors that are mutually interdependent. In addition, those factors that explained patterning at small scale were also those that contributed to large-scale heterogeneity. Thus, the bedrock profile was also a complex system with emergent

properties, or those patterns that arise from the local interactions of individual components.

A primary reason may be that these interactions occur in areas where mycorrhizae mineralize inorganic nutrients, such as N and P, organic N during the decomposition of organic materials within substrates, or bind these minerals during nutrient assimilation (Allen, 1991). Summer or winter changes in bedrock microbial communities may release organic N that becomes available to mycorrhizae. The emphasis on N reflects the N-limitation present in many California soils. Mycorrhizae were also polarized toward resource-rich areas that included Ca, Mg and K. Such minerals are usually derived from biogeochemical weathering of substrates. However, the hyphae-Ca relationship may indicate the accumulation of Ca as Ca oxalates that can be produced by fungal taxa (Cromack et al., 1979). A second reason may be that the weathered bedrock functions as both a water store (Anderson et al., 1995; Hubbert et al., 2001a) that compensates for the uneven supply of water to the whole root system (Robinson, 1996), and a reservoir of mineral nutrients, especially that can be accessed to maintain net productivity. Thus, hyphal proliferation with depth possibly reflects a compromise between water and nutrient availability.

Based on the degree of non-random spatial variability in fungi and resources on both the vertical and horizontal dimensions, we suggest that hyphal foraging in a heterogeneous bedrock environment may be important to the water and nutrient balance of plants in the chaparral community. Whether the patterns and properties of resource acquisition are due to root distribution, shifts in the distribution, diversity and abundance of fungal taxa, or to a physiological acclimation of a constant set of fungal taxa, the linkage between the ecophysiological characteristics of the fungal and plant biota and a higher-level ecosystem processes is novel.

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